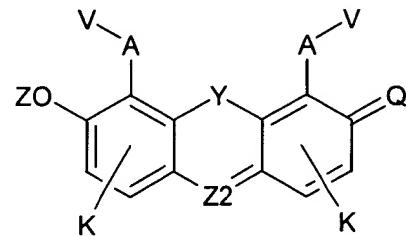
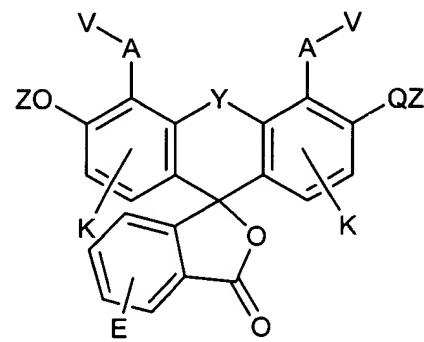


In the claims:

1. (currently amended) A fluorescein-based ligand, comprising a ligand having one of the following structures:

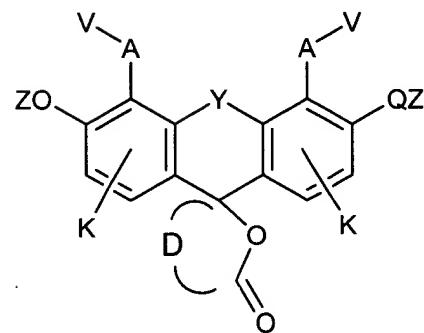


I



II

or



III

wherein, independently for each occurrence:

A is one of the following, with the hydrogen atoms optionally substituted: -CH<sub>2</sub>-, -C(=O)-, -C(=S)-, -CH<sub>2</sub>CH<sub>2</sub>-, -CH<sub>2</sub>C(=O)-, or -CH<sub>2</sub>C(=S)- or [-C(H)=-]];

Z is hydrogen or any hydroxyl-protecting group;

Q is O, S or Se;

K is optionally one or more of the following substituents of the indicated aromatic ring: aliphatic, alkyl, aralkyl, alkenyl, alkynyl, aryl, amine, acyl, acyloxy, acylamino, amide, alkylthio, carbonyl, alkoxy, sulfonate, sulfate, sulfamoyl, sulfonyl, sulfoxido, selenoalkyl, nitro, halogen, sulfhydryl, cyano, hydroxyl, carbamoyl, sulfonyl and trifluoromethyl;

V is amino, amido, nitro, nitroso, amino alcohol, nitrile, imino, isonitrile, cyanate, isocyanate, phosphate, phosphonate, phosphite, phosphine, phosphine oxide, phosphorothioate, phosphoramidate, phosphonamidite, hydroxyl, carbonyl, aldehyde, ketone, ether, carbamoyl, thiol, sulfide, thiocarbonyl, thioether, mercaptan, sulfonic acid, sulfoxide, sulfate, sulfonate, sulfone, sulfonamide, sulfamoyl, sulfinyl, or heterocyclyl a chemical moiety comprising (i) at least three Lewis bases that are capable of forming a tridentate chelating agent, wherein at least one of said three Lewis bases is a ring heteroatom of a heterocyclic group, or (ii) a secondary nitrogen atom doubly bonded to a carbon atom of A to form an imine, wherein said secondary nitrogen atom is capable of forming a bidentate chelating agent with the oxygen atom of OZ or Q;

Y is O, S, Se, NR, or C(CH<sub>3</sub>)<sub>2</sub>, wherein R is an alkyl and R and the methyl groups of C(CH<sub>3</sub>)<sub>2</sub> are optionally substituted;

Z2 is N, HOOCCH<sub>2</sub>CH<sub>2</sub>C-, HOOC-CH=CH-C-, (2-carboxyphenyl)-C-, or (2-sulfophenyl)-C-, wherein for said (2-carboxyphenyl)-C- and (2-sulfophenyl)-C-, said phenyl moiety is optionally substituted with one or more E, and wherein for said HOOCCH<sub>2</sub>CH<sub>2</sub>C- and HOOC-CH=CH-C-, said hydrogen atoms of said -CH<sub>2</sub>- [[']s]] and -CH= [[']s]] moieties are optionally substituted and said carbonyls are optionally a carbonyl other than carboxylic acid;

E is optionally one or more of the following substituents of the indicated aromatic ring: aliphatic, alkyl, aralkyl, alkenyl, alkynyl, aryl, amine, acyl, acyloxy, acylamino, amide, alkylthio, carbonyl, alkoxy, sulfonate, sulfate, sulfamoyl, sulfonyl, sulfoxido, selenoalkyl, nitro, halogen, sulfhydryl, cyano, hydroxyl, carbamoyl, sulfonyl and trifluoromethyl; and

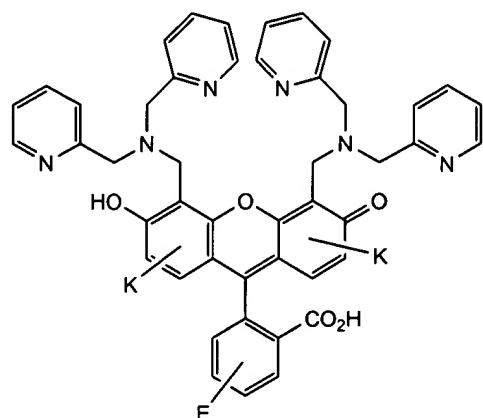
D is -CH<sub>2</sub>CH<sub>2</sub>- or -CH=CH-, wherein said hydrogen atoms are optionally substituted.

2. **(previously presented)** The fluorescein-based ligand of claim 1, wherein A is -CH<sub>2</sub>-, optionally substituted, Y is O, and Q is O.
3. **(previously presented)** The fluorescein-based ligand of claim 2, wherein: said ligand has formula I or II; K is optionally one or more of the following substituents of the indicated aromatic ring: halogen; Z2 is (2-carboxyphenyl)-C-; and E is optionally one or more of the following substituents of the indicated aromatic ring: amino, nitro, carbonyl and halogen.
4. **(previously presented)** The fluorescein-based ligand of claim 3, wherein K is present as halogen in either or both of the 2' and 7' positions of said ligand, and wherein E is not present.
5. **(currently amended)** The fluorescein based-ligand of claim 1, wherein: Q is O; Y is O; and V is amino ~~comprises at least three Lewis bases that are capable of forming a tridentate chelating agent, wherein at least one of said three Lewis bases is a nitrogen ring heteroatom of a heterocyclic group.~~
6. **(currently amended)** The fluorescein-based ligand of claim 1, wherein: Y is O; Q is O; Z is H; and V is amino ~~comprises at least three Lewis bases that are capable of forming a tridentate chelating agent, wherein at least one of said three Lewis bases is a nitrogen ring heteroatom of a heterocyclic group, and wherein said tridentate chelating agent is capable of forming a tetridentate chelating agent with the oxygen atom of OZ or Q.~~
7. **(previously presented)** The fluorescein-based ligand of claim 3, wherein K is present at both the 2' and 7' positions of said ligand.
8. **(previously presented)** The fluorescein-based ligand of claim 4, wherein: said ligand has formula I or II; K is optionally one or more of the following substituents of the indicated aromatic ring: halogen; Z2 is (2-carboxyphenyl)-C-; and E is optionally one or more of the following substituents of the indicated aromatic ring: amino, nitro, carbonyl and halogen.

9. (previously presented) The fluorescein-based ligand of claim 8, wherein K is present in one or both of the indicated aromatic rings as halogen.

10. (previously presented) The fluorescein-based ligand of claim 8, wherein E is present in said ligand as either one carbonyl or one amino.

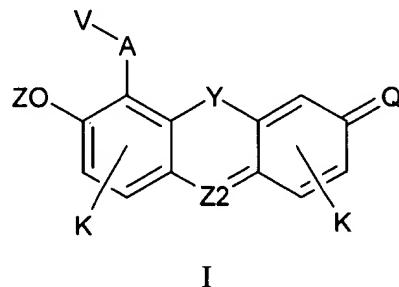
11. (previously presented) The fluorescein-based ligand of claim 1, wherein said ligand has the following structure:



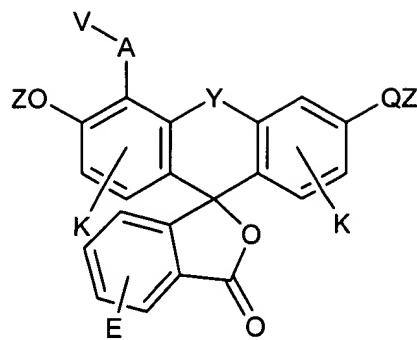
wherein K is optionally one or more of the following substituents of the indicated aromatic ring: halogen.

Claims 12-19 (canceled)

20. (currently amended) A fluorescein-based ligand, comprising a ligand having one of the following structures:

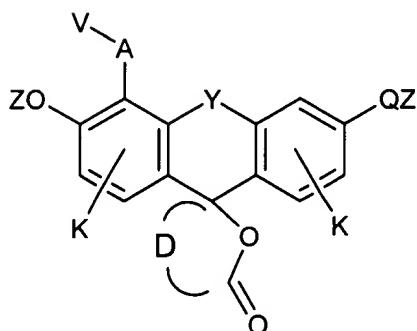


I



II

or



III

wherein, independently for each occurrence:

A is one of the following, with the hydrogen atoms optionally substituted: -CH<sub>2</sub>-; -

C(=O)-, -C(=S)-, -CH<sub>2</sub>CH<sub>2</sub>-; -CH<sub>2</sub>C(=O)-, or -CH<sub>2</sub>C(=S)- or [-C(H)=-]];

Z is hydrogen or any hydroxyl-protecting group;

Q is O, S or Se;

K is optionally one or more of the following substituents of the indicated aromatic ring: aliphatic, alkyl, aralkyl, alkenyl, alkynyl, aryl, amine, acyl, acyloxy, acylamino, amido, alkylthio, carbonyl, alkoxy, ~~sulfonate~~, ~~sulfate~~, ~~sulfamoyl~~, sulfonyl, ~~sulfoxide~~, selenoalkyl, nitro, halogen, sulfhydryl, cyano, hydroxyl, carbamoyl, sulfonyl and trifluoromethyl;

V is amino, amido, nitro, nitroso, amino alcohol, nitrile, imino, isonitrile, cyanate, isocyanate, phosphate, phosphonate, phosphite, phosphine, phosphine oxide, phosphorothioate, phosphoramidate, phosphonamidite, hydroxyl, carbonyl, aldehyde, ketone, ether, carbamoyl, thiol, sulfide, thiocarbonyl, thioether, mercaptan, sulfonic acid,

sulfoxide, sulfate, sulfonate, sulfone, sulfonamide, sulfamoyl, sulfinyl, or heterocyclyl  
~~chemical moiety comprising (i) at least three Lewis bases that are capable of forming a tridentate chelating agent, wherein at least one of said three Lewis bases is a ring heteroatom of a heterocyclic group, or (ii) a secondary nitrogen atom doubly bonded to a carbon atom of A to form an imine, wherein said secondary nitrogen atom is capable of forming a bidentate chelating agent with OZ;~~

Y is O, S, Se, NR, or C(CH<sub>3</sub>)<sub>2</sub>, wherein R is an alkyl and R and the methyl groups of C(CH<sub>3</sub>)<sub>2</sub> are optionally substituted;

Z2 is N, HOOCCH<sub>2</sub>CH<sub>2</sub>C-, HOOC-CH=CH-C-, (2-carboxyphenyl)-C-, or (2-sulfophenyl)-C-, wherein for said (2-carboxyphenyl)-C- and (2-sulfophenyl)-C-, said phenyl moiety is optionally substituted with one or more E, and wherein for said HOOCCH<sub>2</sub>CH<sub>2</sub>C- and HOOC-CH=CH-C-, said hydrogen atoms of said -CH<sub>2</sub>- [[']s]] and -CH= [[']s]] ~~moieties are optionally substituted and said carbonyls are optionally a carbonyl other than carboxylic acid;~~

E is optionally one or more of the following substituents of the indicated aromatic ring: ~~aliphatic~~, alkyl, ~~aralkyl~~, alkenyl, alkynyl, ~~aryl~~, amine, acyl, acyloxy, acylamino, ~~amide~~, alkylthio, carbonyl, alkoxy, ~~sulfonate, sulfate, sulfamoyl, sulfonyl, sulfoxide, selenoalkyl, nitro, halogen, sulfhydryl, cyano, hydroxyl, carbamoyl, sulfonyl and trifluoromethyl; and~~

D is -CH<sub>2</sub>CH<sub>2</sub>- or -CH=CH-, wherein said hydrogen atoms are optionally substituted.

21. **(previously presented)** The fluorescein-based ligand of claim 20, wherein A is -CH<sub>2</sub>-, optionally substituted, Y is O, and Q is O.
22. **(previously presented)** The fluorescein-based ligand of claim 21, wherein: said ligand has formula I or II; K is optionally one or more of the following substituents of the indicated aromatic ring: halogen; Z2 is (2-carboxyphenyl)-C-; and E is optionally one or more of the following substituents of the indicated aromatic ring: amino, nitro, carbonyl and halogen.

23. **(previously presented)** The fluorescein-based ligand of claim 22, wherein K is present as halogen in either or both of the 2' and 7' positions of said ligand, and wherein E is not present.

24. **(currently amended)** The fluorescein based-ligand of claim 20, wherein: Q is O; Y is O; and V is amino ~~comprises at least three Lewis bases that are capable of forming a tridentate chelating agent, wherein at least one of said three Lewis bases is a nitrogen ring heteroatom of a heterocyclic group.~~

25. **(currently amended)** The fluorescein-based ligand of claim 20, Y is O; Q is O; Z is H; and V is amino ~~comprises at least three Lewis bases that are capable of forming a tridentate chelating agent, wherein at least one of said three Lewis bases is a nitrogen ring heteroatom of a heterocyclic group, and wherein said tridentate chelating agent is capable of forming a tetridentate chelating agent with OZ.~~

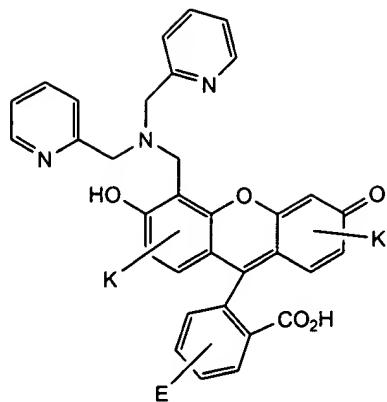
26. **(previously presented)** The fluorescein-based ligand of claim 22, wherein K is present at both the 2' and 7' positions of said ligand.

27. **(previously presented)** The fluorescein-based ligand of claim 24, wherein: said ligand has formula I or II; K is optionally one or more of the following substituents of the indicated aromatic ring: halogen; Z2 is (2-carboxyphenyl)-C-; and E is optionally one or more of the following substituents of the indicated aromatic ring: amino, nitro, carbonyl and halogen.

28. **(previously presented)** The fluorescein-based ligand of claim 27, wherein K is present in one or both of the indicated aromatic rings as halogen.

29. **(previously presented)** The fluorescein-based ligand of claim 27, wherein E is present in said ligand as either one carbonyl or one amino.

30. **(previously presented)** The fluorescein-based ligand of claim 20, wherein said ligand has the following structure:

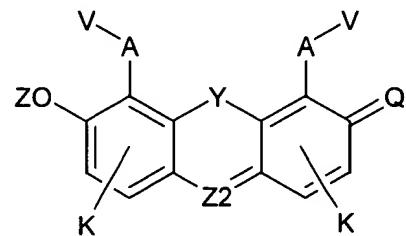


wherein K is optionally one or more of the following substituents of the indicated aromatic ring:  
halogen.

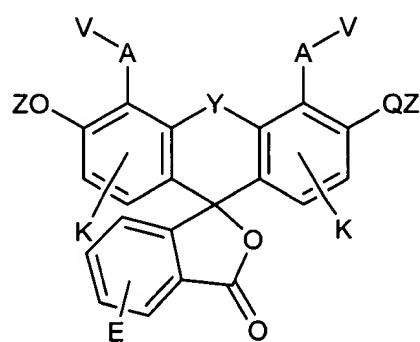
Claims 31-47 (canceled)

48. (currently amended) A diagnostic kit for a metal ion, comprising:

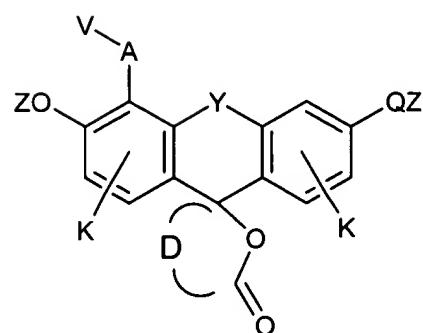
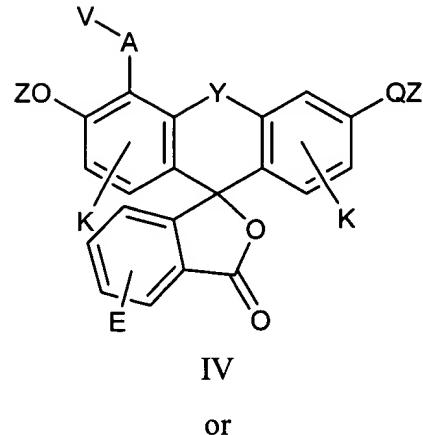
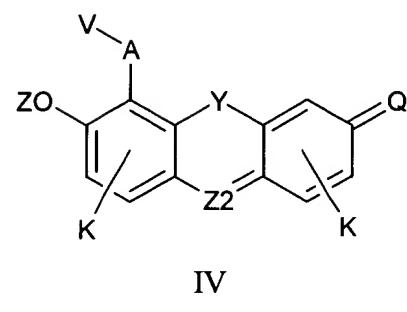
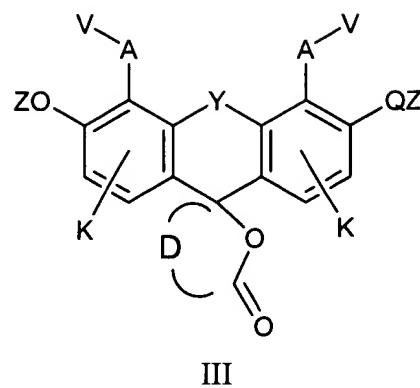
a. A fluorescein-based ligand comprising one of the following structures:



I



II



wherein, independently for each occurrence:

A is ~~one of the following, with the hydrogen atoms optionally substituted:~~ -CH<sub>2</sub>-, -C(=O)-, -C(=S)-, -CH<sub>2</sub>CH<sub>2</sub>-, -CH<sub>2</sub>C(=O)-, or -CH<sub>2</sub>C(=S)- or [-C(H)=-]];;

Z is hydrogen or any hydroxyl-protecting group;

Q is O, S or Se;

K is optionally one or more of the following substituents of the indicated aromatic ring: ~~aliphatic, alkyl, aralkyl, alkenyl, alkynyl, aryl, amine, acyl, acyloxy, acylamino, amide, alkylthio, carbonyl, alkoxyl, sulfonate, sulfate, sulfamoyl, sulfonyl, sulfoxide, selenoalkyl, nitro, halogen, sulphydryl, cyano, hydroxyl, carbamoyl, sulfonyl and trifluoromethyl;~~

V ~~amino, amido, nitro, nitroso, amino alcohol, nitrile, imino, isonitrile, cyanate, isocyanate, phosphate, phosphonate, phosphite, phosphine, phosphine oxide, phosphorothioate, phosphoramidate, phosphonamidite, hydroxyl, carbonyl, aldehyde, ketone, ether, carbamoyl, thiol, sulfide, thiocarbonyl, thioether, mercaptan, sulfonic acid, sulfoxide, sulfate, sulfonate, sulfone, sulfonamide, sulfamoyl, sulfinyl, or heterocyclyl is a chemical moiety comprising (i) at least three Lewis bases that are capable of forming a tridentate chelating agent, wherein at least one of said three Lewis bases is a ring heteroatom of a heterocyclic group, or (ii) a secondary nitrogen atom doubly bonded to a carbon atom of A to form an imine, wherein said secondary nitrogen atom is capable of forming a bidentate chelating agent with the oxygen atom of OZ or Q;~~

Y is O, S, Se, NR, or C(CH<sub>3</sub>)<sub>2</sub>, wherein R is an alkyl and R and the methyl groups of C(CH<sub>3</sub>)<sub>2</sub> are optionally substituted;

Z2 is N, HOOCCH<sub>2</sub>CH<sub>2</sub>C-, HOOC-CH=CH-C-, (2-carboxyphenyl)-C-, or (2-sulfophenyl)-C-, wherein for said (2-carboxyphenyl)-C- and (2-sulfophenyl)-C-, said phenyl moiety is optionally substituted with one or more E, and wherein for said HOOCCH<sub>2</sub>CH<sub>2</sub>C- and HOOC-CH=CH-C-, said hydrogen atoms of said -CH<sub>2</sub>- [']s]] and -CH= [']s]] ~~moieties are optionally substituted and said carbonyls are optionally a carbonyl other than carboxylic acid;~~

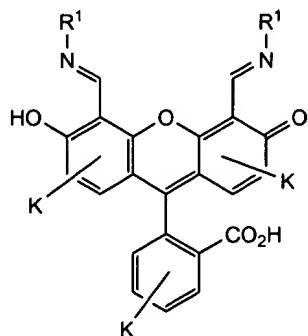
E is optionally one or more of the following substituents of the indicated aromatic ring: ~~aliphatic~~, alkyl, ~~aralkyl~~, alkenyl, alkynyl, ~~aryl~~, amine, acyl, acyloxy, acylamino, ~~amide~~, alkylthio, carbonyl, alkoxy, ~~sulfonate~~, ~~sulfate~~, ~~sulfamoyl~~, sulfonyl, ~~sulfoxide~~, ~~selenoalkyl~~, nitro, halogen, sulphydryl, cyano, hydroxyl, carbamoyl, sulfonyl and trifluoromethyl; and

D is -CH<sub>2</sub>CH<sub>2</sub>- or -CH=CH-, wherein said hydrogen atoms are optionally substituted; and

b. Instructions for using said ligand to detect a metal ion in a sample.

49. (canceled)

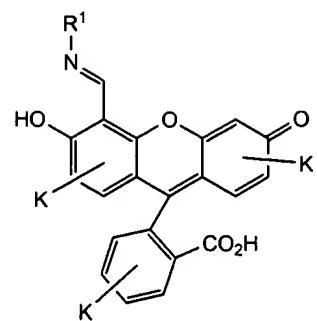
50. (new) The fluorescein-based ligand of claim 1, wherein said ligand has the following structure:



wherein

R<sup>1</sup> represents optionally substituted aliphatic, alkyl, aralkyl, alkenyl, alkynyl, aryl or heterocyclyl.

51. (new) The fluorescein-based ligand of claim 20, wherein said ligand has the following structure:



wherein

$R^1$  represents optionally substituted aliphatic, alkyl, aralkyl, alkenyl, alkynyl, aryl or heterocyclyl.